Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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State of the Science Review

Are saliva and deep throat sputum as reliable as common respiratory specimens for SARS-CoV-2 detection? A systematic review and meta-analysis

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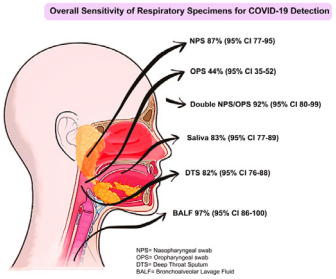

Key Words:
COVID-19
Sputum
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Oropharyngeal swab
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RT-PCR diagnostic test

Objective: The COVID-19 pandemic raises an urgent need for large-scale control through easier, cheaper, and safer diagnostic specimens, including saliva and sputum. We aimed to conduct a systemic review and meta-analysis on the reliability and sensitivity of SARS-CoV-2 detection in saliva and deep throat sputum (DTS) compared to nasopharyngeal, combined naso/oropharyngeal, and oropharyngeal swabs.

Methods: This systematic review and meta-analysis was performed according to the PRISMA statement. The inclusion criteria were studies that specifically assessed a sample of saliva or DTS with at least one other respiratory specimen in patients with COVID-19 infection, based on RT-PCR tests. The DerSimonian-Laird bivariate random-effects model analysis performed using STATA software with the "metaprop" package.

Results: From 1598 studies, we retrieved 33 records, of which 26 studies were included for quantitative analysis. We found an overall sensitivity of 97% (95% confidence interval [CI], 86-100) for bronchoalveolar lavage fluid, 92% (95% CI, 80-99) for double naso/oropharyngeal swabs, 87% (95% CI, 77-95) for nasopharyngeal swabs, 83% (95% CI, 77-89) for saliva, 82% (95% CI, 76-88) for DTS, and 44% (95% CI, 35-52) for oropharyngeal swabs among symptomatic patients, respectively. Regardless of the type of specimens, the viral load and sensitivity in the severe patients were higher than mild and in the symptomatic patients higher than asymptomatic cases.

Conclusions: The present review provides evidence for the diagnostic value of different respiratory specimens and supports saliva and DTS as promising diagnostic tools for first-line screening of SARS-CoV-2 infection. However, the methods of sampling, storing, and laboratory assay need to be optimized and validated before introducing as a definitive diagnosis tool. Saliva, DTS, and nasopharyngeal swab showed approximately similar results, and sensitivity was directly related to the disease severity. This review revealed a relationship between viral load, disease severity, and test sensitivity. None of the specimens showed appropriate diagnostic sensitivity for asymptomatic patients.

INTRODUCTION

COVID-19 disease, caused by the SARS-CoV-2, is a severe infection causing morbidity and mortality worldwide. More than 200 countries and territories are affected, more than 64,000,000 are infected, and approximately 1,500,000 deaths are reported by December 2020.[1] It

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has now been acknowledged that early detection, isolation, and management of infected individuals will play a critical role in stopping the pandemic's further escalation.

Nasopharyngeal swab (NPS) followed by RT-PCR laboratory confirmation is the most recommended diagnostic method for COVID-19 detection.[2] The collection of common respiratory samples such as nasopharyngeal and oropharyngeal specimens require trained medical personnel,[3] which exposes staff to a high risk of infection.[4] While these tests are not always successful at first, shortages of swabs, sample transport media, and personal protective equipment are frequently reported.[3,5] Mass testing requires an increased number of trained personnel at specimen acquisition sites. Also, nasopharyngeal sampling causes discomfort to patients,[4] and there are several contraindications, such as coagulopathy or anticoagulant therapy and significant nasal septum deviation.[3] Considering the high rate of disease transmission and the drawbacks of common respiratory sampling techniques, the use of more flexible, less invasive, and facile specimens for RT-PCR diagnosis tests is crucial.

Despite the heterogenic origin of saliva, it is informative to identify various oral and systemic conditions and viral infections such as severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS).[4,6]

Therefore, since early January 2020, there has been a growing interest in using salivary secretions and deep throat sputum (DTS) as alternatives for common respiratory samples to diagnose COVID-19 infection. The literature has been indicated the possible use of saliva or DTS as a diagnostic specimen for detecting SARS-CoV-2 based on RT-PCR tests.[7-10] Several systematic reviews and meta-analyses aimed to conclude this diagnostic samples' efficacy and compare it to other specimens such as NPS and Oropharyngeal swab (OPS).[7-10] The present comprehensive systematic review and meta-analysis aimed to overcome the limitations of the small sample sized studies and heterogenic outcomes associated with the different clinical course of the disease to estimate the diagnostic sensitivity of different oral and pharyngeal-based specimens and compare the ability and reliability of different respiratory specimens for detection of the SARS-CoV-2.

METHODS

We systematically searched four major databases for this systematic review and meta-analysis (PubMed, Scopus, WoS, and PMC). We also manually searched Google Scholar and preprint archives, references of included studies, cited and citing papers of the relevant studies for relevant results, and sought suggestions from experts to supplement the database searches. We considered original articles (published or pre-print) and conference proceedings without time restriction. (last updated August 2020)

We used the following search terms and their variations: "COVID-19", "SARS-COV-2", "novel coronavirus", "2019 novel coronavirus", "new coronavirus", "diagnosis", "diagnostic", "diagnostic test", "diagnostic assay", "saliva", "sputum", "oral fluid", "oral secretion", "Deep throat saliva", "Deep throat sputum", "oropharyngeal saliva", "Deep throat secretion", and other terms combined with Boolean operators "AND" and "OR".

Eligibility criteria

We included studies in this systematic review and meta-analysis if they met all the following eligibility criteria: (1) records published or under-publishing in scientific journals (including preprint studies); (2) patients diagnosed with or screened for COVID-19; (3) the diagnosis based on RT-PCR method; (4) studies designed to specifically use samples of saliva or oropharyngeal sputum or oral secretion or oral fluids or pharyngeal secretion for quantitative or nonquantitative comparison of diagnostic methods and viral loading in SARS-CoV-2 infected patients; (5) studies that assessed at least 2 respiratory specimens; (6) studies conducted on the previously confirmed COVID-19 patients or compared simultaneously with matched (paired) specimen. Exclusion criteria: (1) publications with no primary outcomes such as reviews, guidelines, and recommendations; (2) publications dated before January and after August 11, 2020.

Study selection and data collection

Two authors (KK and MHA) independently screened titles and abstracts of all publications identified through the literature search, reviewed potentially eligible full-text papers using the predefined criteria, extracted data from included studies, and assessed methodological quality. Discrepancies were resolved through consensus between two researchers. Unsolved cases were referred to a third reviewer, and duplicate studies were excluded. The following data were extracted and calculated from the text and tables and transferred to the preconstructed data extraction form: author's name, place of study, method of diagnosis, sampling technique, matched or reference specimen, population size, viral load, and the following outcome parameters: numbers of total, positive and negative saliva or DTS tests regarding disease status and numbers of total, positive and negative NPS, OPS, double NPS/OPS regarding disease status.

Risk of bias, applicability assessment, and protocol registration

Two reviewers (KK and MHA) evaluated independently the risk of bias in each study using the "Diagnostic Precision Study Quality Assessment Tool" (QUADAS-2) recommended by the Cochrane Collaboration. Our assessment consisted of evaluating the risk of bias in 4 domains: (1) patient selection, (2) conduct and interpretation of the index test based on saliva or deep throat sputum, (3) reference standard or matched test based on NPS, OPS, double NPS/OPS, and (4) flow and timing and three first domains for applicability concerns. The assessment was performed using the Review Manager Software version 5.4 (Cochrane Collaboration, London, UK).[11,12] This study is reported according to PRISMA guidelines.[13]

Data analysis

Regardless of the number of tests performed for a patient, each test result is crucial to assess different specimens' sensitivity. Since the difference in the number of tests between sample-based and patient-based reports was small, we included both types in the analysis. All case reports presented a single participant's data were excluded. One of the following methods have been used to confirm the COVID-19 infection in the studies included for quantitative or non-quantitative assessment of oral or retropharyngeal specimens:

1. Study was performed on patients who had been confirmed for COVID-19 infection by RT-PCR.
2. Diagnosis was based on a reference test, NPS or OPS or double NPS/OPS, collected in parallel with saliva or sputum (matched/paired sampling).
3. Infection was confirmed based on pooled event rates (positive and negative results) of saliva/ DTS and other respiratory specimens.

In the present study, to reduce the heterogeneity in the diagnosis methods, the second type was changed to the third type based on the pool of positive and negative saliva/DTS results and the reference/matched sample. Sensitivity, defined as the probability that a test result will be positive when the disease exists (true positive rate), was calculated as TP/ (TP + FN).

The DerSimonian-Laird bivariate random-effects model analysis was performed with STATA software version 16 (StataCorp, TX) with the "metaprop" package written by Victoria N. Nyaga. For analyses involving studies with a small sample size and sensitivity value too high (toward 1) or low (toward 0), we incorporated the Freeman-Tukey Double Arcsine Transformation method to stabilize the variances by-study confidence intervals.

RESULTS

A common confusion in the studies that use oral & retropharyngeal fluids specimens is the unclear definition of "saliva". To achieve greater consistency in this analysis, we divided studies into 2 main categories: saliva-based and DTS-based studies. DTS contains upper and lower respiratory tract secretions and collected in the same way as recommended for lower respiratory tract sputum. Saliva (oral fluid) sampling includes 2 common techniques: sampling through frequent spitting out (Drooling technique) and direct sampling from the oral fluid pool. In general, the drooling technique should not be considered pure saliva sampling because bronchoalveolar secretion, nasopharyngeal discharge, and other intraoral substances are added to the saliva. Direct saliva sampling includes using saliva sampling kits, instruments, and swabs to collect saliva from the salivary pool under the tongue tip near the orifice of major salivary glands, exactly posterior to the lower anterior teeth. In this study, saliva and oral fluids and oral secretion were used interchangeably. All extracted studies that mentioned sampling of oral fluid or sampling without referring to oropharyngeal secretion were included in the salivary group. Deep throat (upper respiratory) sputum sampling is performed by throat clearing and coughing up and out the secretion and sputum of the retropharynx. All extracted studies sampled deep throat specimens with or without coughing were included in the DTS group. However, a few studies that referred to retropharyngeal sampling without cough and sputum were analyzed separately (DT-secretion).

From 1,598 articles retrieved from the initial search, 57 saliva/DTS-related studies were identified in the title and abstract screening after duplicate removal. According to inclusion criteria, 33 studies were meet the review's aims. The "Saliva" group included 19 articles,[14-32] and the "DTS" group included 14 articles.[33-46] (Supplementary Material Tables S1 and S2) For the quantitative analyses (meta-analysis), 16 and 10 trials in the Saliva and DTS groups were included, respectively.[14-29,33-42] The remaining studies, which did not report quantitative information, were only systematically reviewed.[30-32,43-46] (Supplementary Material Fig S1)

Results of the "Saliva" group

Seventeen studies provided appropriate quantitative information to calculate the saliva sample sensitivity.[14-30] However, the Chen et al study, which was the only study using a different collection method to obtain almost pure saliva, was not included in the statistical analysis.[30] The summary of diagnostic results from saliva and other respiratory specimens is listed in Table 1. The remaining articles were included in systematic reviews.[31,32] Ten studies assessed the diagnostic test results in the previously laboratory-confirmed COVID-19 patients,[14-21,30,31] while eight studies used the pooled event rates of salivary test results with another specimen to obtain the sensitivity.[22-29] (Supplementary Material Table S1)

Fourteen studies performed sampling concurrently from saliva and other areas (matched samples).[14-17,20,22-30] Four studies reported nonmatched sample results.[18,19,21,31] NPS,[14-19,23,25,27,28,31] OPS,[21,22,30] combined Naso/oropharyngeal swabs[20,22,24,26,29] were used as a reference or as a matched specimen with saliva sample in 11, 3 and 5 studies, respectively.

In the "Saliva" group, most studies have used the drooling technique to collect saliva.[15-17,22-25,27-29] Two studies collected saliva after pooling in the mouth,[14,18] and Two studies have used swab sampling,[16,31] one study utilized a saliva-collecting kit,[25] and one study has used one of the following techniques: drooling, pipette or swab for saliva sampling.[16] however, 5 studies did not describe the collection method at all.[19-21,26,32]

Two, 5, and 8 studies evaluated patients in severe,[15,16] severe in combination with mild to moderate,[17,19,22,29,30], and mild to moderate[14,18,20,23-25,27,28] conditions, respectively. Also, 12, 2, and one studies included symptomatic,[14-19,22-27] symptomatic combined with asymptomatic,[20,31] and asymptomatic patients.[21]

Sensitivity and viral load in saliva-based tests

Sixteen eligible studies, including 1052 patients with 1056 salivary tests based on the previously confirmed patients (859 positive vs 197 negative SARS-CoV-2 samples), provided quantitative analysis information. Most of the studies reported patient-based results.[14,16-30] However, Wyllie et al conducted a sample-based study.[15] The saliva test's sensitivity possessed a wide range from 25%[31] to 100%[16] (Table 1). The saliva test's overall sensitivity in symptomatic patients showed 83% (95% CI = 77-89; I^2 = 79.04%) (Fig 1). However, it decreased to 81% (95% CI = 74-87; I^2 = 81.26%) when asymptomatic patients were included. (Supplementary Material Fig S2) According to the disease confirmation method, the saliva test sensitivity in symptomatic patients was calculated as 83% (95% CI = 68-95; I^2 = 85.23%) for studies based on previously confirmed patients and 84% (95% CI = 77-90; I^2 = 65.21%) for studies based on pooled event rates. (Supplementary Material Figure S3)

Salivary sensitivity and viral load regarding disease severity

The highest sensitivity (100%) for saliva specimen test among all studies was reported by Azzi et al. from 25 severe patients[16] (Table 1). For severe patients, pooled sensitivity assessed 90% (95% CI = 76-99; I^2= 72.43%) (Fig 1). Studies in which the cases were all mild to moderate patients or a small number were severe, categorized as "mild to moderate" disease subgroup.[14,17,18,20,22-28] In this category, most of the studies reported higher viral load for NPS than saliva specimen[14,17,23,25,27,28,30-32] (Supplementary Material Table S1). In mild to moderate stage patients, pooled sensitivity estimated 81% (95% CI = 73-88; I^2= 80.77%) (Fig 1). Two studies tested asymptomatic patients[20,21] (Table 1). Chau et al. stated lower viral load in saliva than double NPS/OPS in symptomatic and asymptomatic patients, which was statistically significant in asymptomatic individuals.[20] In agreement with them, Kam et al reported a lower viral load in saliva samples than NPS[31] (Supplementary Material Table S1). In asymptomatic patients, pooled sensitivity estimated 46% (95% CI = 27-66). (Supplementary Material Fig S2)

Results related to the "DTS" group

Ten studies provided quantitative information to calculate the sputum specimen sensitivity.[32-42] Other remaining studies were systematically reviewed (Supplementary Material Table S2).[43-46] The summary of diagnostic results from DTS and other respiratory specimens is listed in Table 2. Nine studies evaluated the test sensitivity in patients with previously confirmed infection[32-41] and one study with pooled event rates.[42] Five and six studies performed paired,[33,36,39,40,42] and nonpaired sampling,[34-38,41] respectively. However, one study used both types of samplings[36] (Table 2).

Six studies collected DTS through throat clearing and coughing up/out into a sterile container.[33-35,38,40,42] One study used a throat washing technique to collect deep throat secretion. However, quantitative information was not available for including in meta-analysis.[43] One study used throat gargling with saliva to obtain deep throat secretion.[38] The clearing of the throat without coughing and trough,

Table 1
Summary of diagnostic results from saliva and other respiratory specimens

Study	Method of diagnosis	Matched (Paired) sampling	Disease severity	Saliva test results					Matched/reference test results				
				Total number samples(n)	Positive samples(n)	Negative samples (n)		Sensitivity (%)	Matched / reference sample	Total number of samples	Positive samples(n)	Negative samples (n)	Sensitivity (%)
Williams et al.[14]	Previously confirmed COVID-19 infection	Yes	Mild to moderate	39	33	6		84.6%	NPS	39	N/A	N/A	N/A
Wyllie et al. [15] (Preprint)	Previously confirmed COVID-19 infection	Yes	severe	38	35	3		92%	NPS	38	30	8	79%
Azzi et al. [16]	Previously confirmed COVID-19 infection	Yes	Severe	25	25	0		100%	NPS	25	23	2	92%
Jamal et al. [17]	Previously confirmed COVID-19 infection	Yes	Mild to moderate (70%)/Severe (30%)	91	52	39		57.1%	NPS	91	64	27	70.3%
Helgouachet al. [18] (Preprint)	Previously confirmed COVID-19 infection	No	Mild to moderate	11	8	3		72.7%	NPS	11	11	0	100%
	Pooled event rates		recovery	9	3	6		33.3%		9	8	1	88.9%
Fang et al. [19]	Previously confirmed COVID-19infection	No	Most cases were severe	32	25	7		78.1%	NPS	32	29	3	90.6%
Chau et al.[20]	Previously confirmed COVID-19infection	Yes	Symptomatic(mild to moderate)	16	13	3	81%	Overall 74%	Double NPS/OPS	17	17	0	100% Overall83.3%
			Asymptomatic	11	7	4	64%			13	8	5	62%
Bosworth et al. [21]	Previously confirmed COVID-19infection	No	Asymptomatic	15	5	10		33%	OPS	15	N/A	N/A	N/A
Contreras et al. [22] (Preprint)	Pooled event rates	Yes Paired with Double NPS & OPS	Most cases were outpatient	34	25	9	73.5%	Overall82.4%	Double NPS & OPS	34	28	6	82.3%
		Paired with OPS	Most cases were outpatient	80	69	11	86%		OPS	80	52	28	65%
Iwasaki et al. [23]	Pooled event rates	Yes	Mild to Moderate	10	9	1		90%	NPS	10	9	1	90%
Pasomsub et al. [24]	Pooled event rates	Yes	symptomatic	21	18	3		85.7%	Double NPS/OPS	21	19	2	90.5%
Becker et al. [25] (Preprint)	Pooled event rates	Yes	Most cases were outpatient	88	61	27		69.3%	NPS	88	87	1	98.9%
Zhu et al.[26]	Pooled event rates	Yes	Most cases were outpatient	457	397	60		86.8%	Double NPS & OPS	457	442	15	96.7%
McCormick et al. [27]	Pooled event rates	yes	Mild to moderate	51	48	3		94.1%	NPS	51	50	1	98%
SoRelle et al. [28] (Preprint)	Pooled event rates	yes	Mild to moderate	23	18	5		78.2%	NPS	23	23	0	100%
Byrne et al. [29] (Preprint)	Pooled event rates	Yes	Most cases were severe	14	11	3		78.5%	Double NPS & OPS	14	13	1	92.8%
			Not Included in Meta-analysis										
Chen et al. [30] (Preprint)	Previously confirmed COVID-19infection	Yes	Mild to moderate	26	1	25	3.8%	Overall12.9%	OPS	26	9	17	34.6% Overall42%
			Severe	5	3	2	60%			5	4	1	80%
Kam et al. [31] (Preprint)	Previously confirmed COVID-19infection	No	Symptomatic(inpatient)/ Asymptomatic	N/A	N/A	N/A	25% to 71.4% on different days of collection		NPS	N/A	N/A	N/A	**N/A**

Note: The Studies from Fang et al. and Byrne et al., which were about half of patients with severe disease, were included in the group of severe patients; The overall sensitivity of the two estimates from the study of Contreras et al. was considered; Estimation of sensitivity in the recovery phase of the study by Helgouach et al. was not included in the analysis; The study by Chen et al., which collected almost pure saliva, was not included in the analysis.
NPS, Nasopharyngeal swab; *OPS*, Oropharyngeal swab; *Double NPS/OPS*, Combined Nasopharyngeal & Oropharyngeal swabs; *N/A*, Not available.

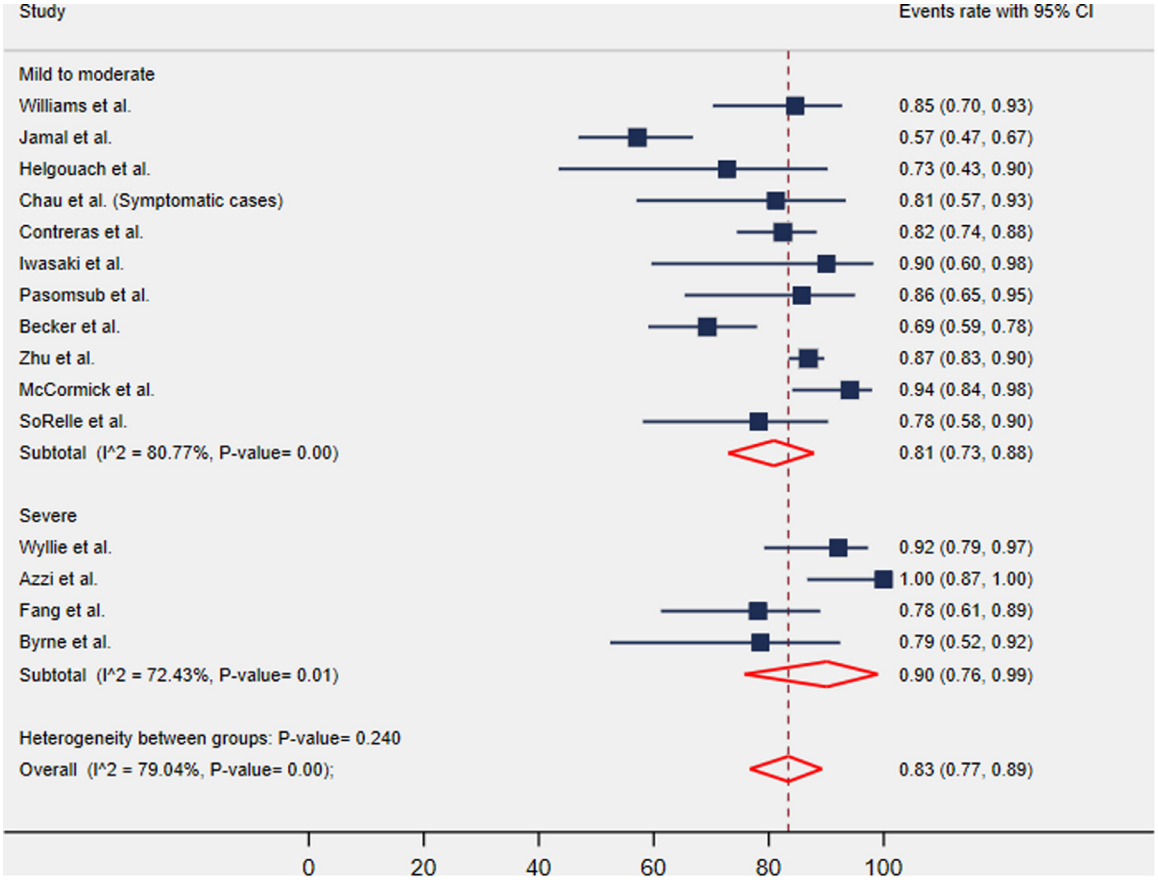

Fig 1. The forest plot of SARS-CoV-2 detection sensitivity of saliva, based on RT-PCR analysis from symptomatic COVID-19 patients regarding the disease's severity.

making the noise of "Kruuua" used to collect posterior oropharyngeal secretion in another study.[39] In the remaining other studies, the technique of sputum sampling was unavailable.[36,37,41] Patients in severe,[34] severe in combination with mild to moderate,[33,35-41] and mild to moderate[42] conditions were evaluated in included studies. Nine symptomatic[33-41] and one symptomatic combined with asymptomatic patients[42] studies were evaluated, respectively.

Sensitivity and viral load in DTS-based tests

Ten eligible studies, including 668 patients with 1034 specimens (759 positive vs 275 negative SARS-CoV-2 samples) from DTS or retropharyngeal secretion, provided quantitative information for sensitivity assessment. However, 379 cough-free samples were collected. Ten and 4 studies reported patient-based[33,36,38,40-42] and sample-based results,[34,35,37,39] respectively. Among all the studies, the sputum specimen's sensitivity was ranged from 53% [36] to 91.6% [34]. (Table 2) The DTS overall sensitivity in symptomatic patients showed 82% (95% CI = 76-88; I^2 = 67.57%) (Fig 2). However, it decreased to 79% (95% CI = 72-85; I^2 = 80.46%) when studies with cough-free secretion were included. The sensitivity of cough-free samples from deep throat secretion was 64.37%.

DTS test sensitivity and viral load regarding disease severity

The highest sensitivity was in studies with severe patients when all cases,[34,37] or about half of the patients were severe[35] (Table 2). Wang et al reported the highest sensitivity rate (91.6%) for the DTS specimen for 12 severe patients.[34] Wang to et al, in a quantitative viral load study,[35] reported higher viral load in severe cases than mild cases. Yu Xia et al found a close relation between viral load and the severity of the disease so that the viral load was higher in severe cases and in patients who became severe during hospitalization.[45] Yu Fengting et al and Pan et al reported the highest viral load early after symptom onset and in severe patients.[41,44] Focusing on severe patients, pooled sensitivity assessed 87% (95% CI = 77-95) (Fig 2).

Studies in which the study population were all mild to moderate patients or a small number were severe, categorized as mild to moderate. Kojima et al, Chen et al, and Wang et al reported higher viral load in NPS than DTS.[33,36,42] (Supplementary Material Table S2) In the only available study comparing viral load in saliva and sputum samples, Yoon et al. reported higher viral load for sputum.[32] Focusing on mild to moderate patients, pooled sensitivity was estimated as 80% (95% CI = 73-87; I^2 = 76.65%) (Fig 2).

Overall sensitivity of other matched-specimens

Altogether, some studies provided quantitative information of NPS[15-19,23,25,27,28,33,36,37,39,42], OPS,[22,30,36,37,40,41] double NPS/OPS[20,22,24,26,29,38] and BALF[36,37] with saliva or DTS specimens, respectively (Table 1 and 2).

Several studies provided quantitative information for NPS sensitivity assessment from critically ill[15,16,19,37] mild to moderate,[17,18,23,25,27,28,33,36,37,39,42] and asymptomatic[42] patients, respectively. Overall NPS sensitivity is estimated 87% (95% CI = 77-95; I^2 = 93.33%). Pooled sensitivity was estimated 83% (95% CI = 73-91, I^2 = 55.10%) regarding the patients in severe stages. Also, for patients in mild to moderate stages, pooled sensitivity was estimated at 88% (95% CI = 76-97; I^2 = 94.97%). Overall double NPS/OPS sensitivity was

Table 2
Summary of diagnostic results from deep throat sputum (DTS) and other respiratory specimens

Study	Method of diagnosis		Matched (Paired) sampling	Disease severity	Deep throat Sputum test results						Matched/ reference test results					
					Total number of samples (n)	positive samples (n)	Negative samples (n)		Sensitivity (%)		Matched/ reference sample	Total number of samples	Positive samples (n)	Negative samples (n)	Sensitivity (%)	
Chen et al. [33]	laboratory-confirmed COVID-19infection		Yes	Hospitalized (Most mild to moderate)	58	52	6		89.7%		NPS	58	55	3	94.8%	
Wang to et al. [34]	laboratory-confirmed COVID-19infection		No	Severe cases	12	11	1		91,6%		NPS	N/A	N/A	N/A	N/A	
Wang to et al. [35]	laboratory-confirmed COVID-19infection		No	Hospitalized/ 43.5% (10patients) severe	23	20	3		87%		NPS	N/A	N/A	N/A	N/A	
Wang et al. [36]	laboratory-confirmed COVID-19infection	Yes/No	Non-matched	Inpatient/ 20% were severe cases	104	75	29	72%	Overall 70%		NPS	8	5	3	63%	Overall NPS
											OPS	398	126	272	32%	78.5%;
											BALF Severe cases	15	14	1	93%	Overall
				matched	Inpatient/ 20% were severe cases	13	7	6	53%		NPS	6	6	0	100%	OPS 33.9%
			OPS								18	15	3	83%		
Yang et al. [37] (Preprint)	laboratory-confirmed COVID-19infection (the results of days 0-14 were considered)		No	Severe17%	27	23	4		85.1%	Overall80%	NPS	62	45	17	72.5%	Overall
											OPS	56	30	26	53.5%	NPS 64.7%;
											BALF	12	12	0	100%	Overall
				Mild to Moderate 83%	88	69	19		78.4%		NPS	383	243	140	63.4%	OPS 53.1%
											OPS	102	54	48	52.9%	
Lai et al.[38]	laboratory-confirmed COVID-19infection	No	Deep throat saliva (secretion)	Mild to Moderate	150	103	47		68.7%		Double NPS/OPS	309	250	59		80.9%
			Sputum		104	93	11		89.4%							
Ying Wong et al. [39]	laboratory-confirmed COVID-19infection	Yes	Posterior oropharyngeal saliva (secretion)	Mild to Moderate	229	141	88		61.6%		NPS	229	122	107		53.3%
Lin et al.[40]	laboratory-confirmed COVID-19infection		Yes	Most mild to moderate	52	40	12		76.9%		OPS	52	23	29		44.2%
Yu Fengting et al. [41]	laboratory-confirmed COVID-19infection		No	Most mild to moderate	116	80	36		69%		OPS	134	51	83		38.1%
Kojima et al. (Preprint) [42]	Pooled event rates /4 types of samples	Yes	Supervised sampling	Symptomatic (mild to moderate)	21	19	2	90.5%	Overall 90%	Overall 77.5%	NPS Symptomatic (mild to moderate)	21	19	2	90.5%	Overall 79%
				Asymptomatic	8	7	1	87.5%								
			Unsupervised sampling	Symptomatic (mild to moderate)	21	15	6	71.4%	Overall66%		Asymptomatic	8	4	4	50%	
				Asymptomatic	8	4	4	50%								

Note: The overall sensitivity of the two estimates from the study of Wang et al was considered; Sampling under the supervision of health care workers from the study of Kojima et al was included in the estimation
NPS, Nasopharyngeal swab; *OPS*, Oropharyngeal swab; *Double NPS/OPS*, Combined Nasopharyngeal & Oropharyngeal swabs; *BALF*, bronchoalveolar lavage fluid; *N/A*, Not available.

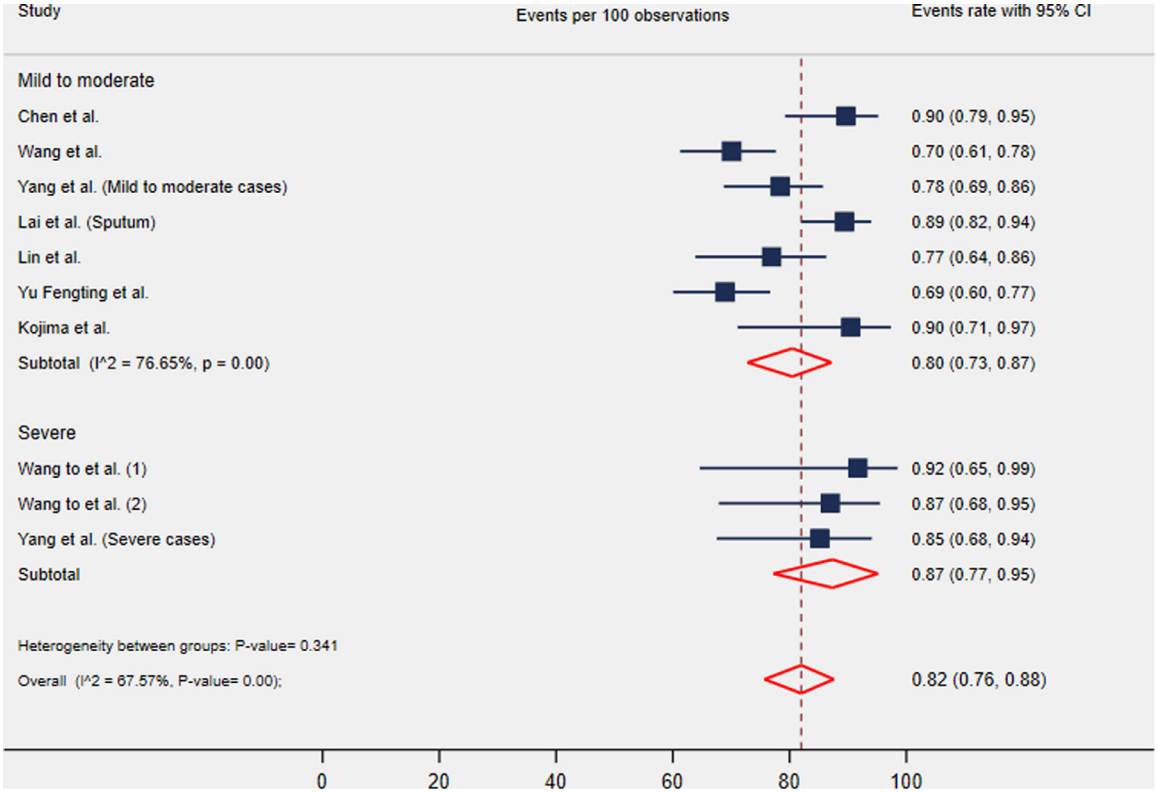

Fig 2. The forest plot of SARS-CoV-2 detection sensitivity of deep throat sputum based on RT-PCR analysis from symptomatic COVID-19 patients regarding the disease's severity.

estimated 89% (95% CI = 78-97; $I^2 = 90.96\%$) (Fig 3). Also, overall OPS sensitivity, estimated 44% (95% CI = 35-52; $I^2 = 77.91\%$). The BALF overall sensitivity was assessed 97% (95% CI = 86-100). (Supplementary Material Fig S4)

Quality assessment

We assessed the risk of bias in 16 and 10 included studies from saliva-based (Fig 4A & Supplementary Material Fig S5A) and DTS-based records (Fig 4B & Supplementary Material Fig S5B). Three saliva-based studies[30-32] and 4 DTS-based studies[43-46] were not evaluated due to the lack of information. Fifty percent of saliva-based and 90% of DTS-based studies were including previously diagnosed patients, enhancing the risk of bias. Hence, selection biases were moderate in saliva-based studies and high in DTS-based studies. There is currently a lack of a reliable reference sample for diagnosing COVID-19 infection, so we assumed NPS, OPS, and combined NPS/OPS as an index test in quality assessment. Overall, 88% of the saliva-based studies and 80% of DTS-based studies have appropriately reported the reference or matched test's results and how they were conducted and interpreted. Around 81% of saliva-based studies and 50% of DTS-based studies conducted matched sampling methods, reducing the risk of bias. Altogether, saliva-based studies were rated as moderate overall bias level (Fig 4A & Supplementary Material Fig S5A), and DTS-based studies were rated as high overall bias level (Fig 4B & Supplementary Material Fig S5B). However, most of the patients included in the saliva group and almost half of the patients included in the DTS group matched the review question and were likely to be diagnosed with the evaluated tests with no significant concerns for the applicability domain.

DISCUSSION

Upper respiratory specimens such as NPS/OPS and lower respiratory specimens such as sputum are recommended specimens for COVID-19 laboratory diagnosis.[2,47] The sensitivity of the tests depends on the type of specimen, sampling procedures, different viral loads in different anatomic sites, the clinical course of the disease, and the variation in viral RNA sequences.[7,48] The NPS is the most recommended and widely used diagnostic specimens,[2,49] but suffers from a lack of an optimal basis for reliable RT-PCR assay.[5,10,48] Up to 29% false-negative results have been reported from upper respiratory samples.[50] In a preprint meta-analysis, the sensitivity of NPS and double NPS/OPS was reported 40%-70%, and 70%-80%, respectively.[8] It appears that a positive test is highly suggestive of true SARS-COV- 2 infection, but a negative test is insufficient to rule out COVID-19.[51] In this regard, failure to diagnose is more consequential in asymptomatic individuals and contributes significantly to further contamination. In addition to the shortage of supply chain,[3,34] the sampling sequences of common respiratory diagnostic specimens such as NPS and OPS are invasive and may induce bleeding, nausea, vomiting, coughing, and sneezing, which these side effects generate aerosols and create a risk of contamination.[4,34,48,52] Clearly, more flexible and less invasive reliable sampling techniques for screening purposes are crucial to informing clinical and public health systems.

Oral fluid (saliva) and DTS are candidates as noninvasive and easy collectible specimens with advantages, including low cost, ease-to-obtain, self-collectability, safety, and no need for highly trained staff.[4] The saliva secreted 90% from the major and rest from the minor salivary glands and mainly consists of 99% water and remaining of electrolytes, mucus, and digestive and protective proteins.[9,53] The fluid collected from the oral cavity known as saliva is a mixture of glandular secretions, gingival crevicular fluid, expectorated surface liquid from the upper & lower airway, oral mucosa and upper airways'

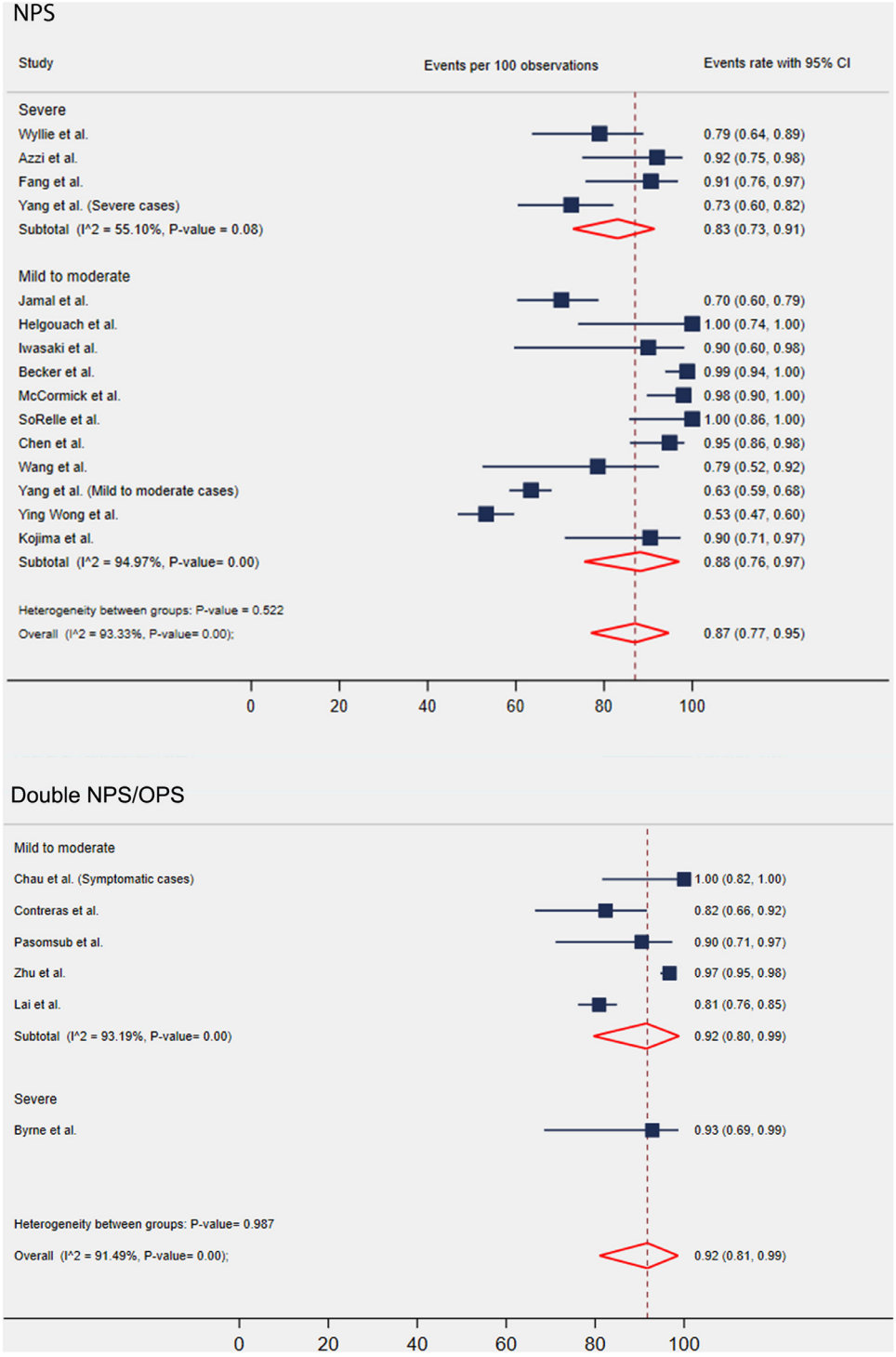

Fig 3. The forest plot of SARS-CoV-2 detection sensitivity of the nasopharyngeal sample and double naso/oropharyngeal samples based on RT-PCR analysis from symptomatic COVID-19 patients regarding the disease's severity.

epithelial and immune cells, and oral microbes and viruses.[9,54] Interestingly, the saliva sample composition is informative for diagnostic purposes to identify various oral and systemic diseases.[6,9] An ideal role for saliva has been reported by isolating proteins, peptides, and even sheds of numerous viruses such as Ebola, Zika, influenza A and B, and the recently emerged coronaviruses responsible for SARS and the MERS.[6,55-57] Following the outbreak of SARS-COV-2 contamination, several studies have indicated saliva's diagnostic role.[14-32] However, the source of SARS-CoV-2 in the saliva is not clearly defined. The most convenient and probable case is the entry of virus-infected secretions from the posterior oropharynx, which is the site of mixing and exchanging secretions and debris in the nasopharynx and lower respiratory tract.[34,35] The second one is salivary gland involvement and direct viral shedding into the saliva. However, precise information is unavailable in this matter.[9] Involvement of epithelial cells lining of salivary gland ducts, as an early target for SARS-

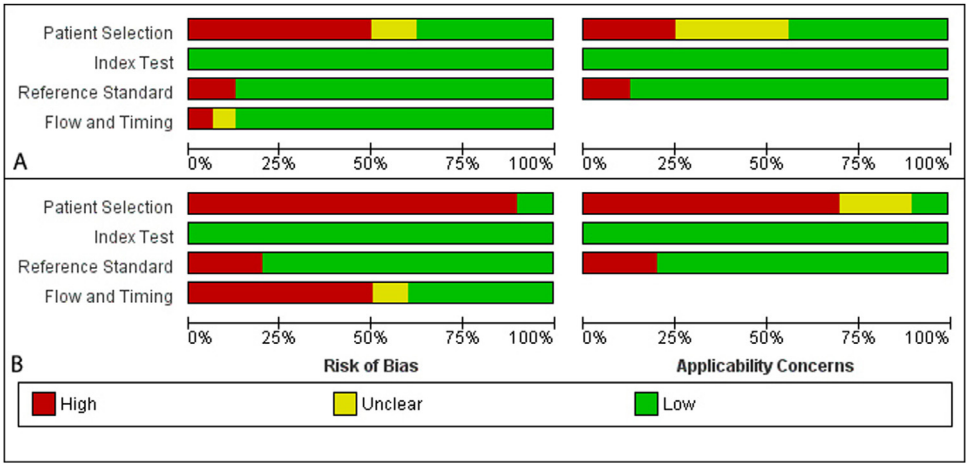

Fig 4. (A) Summary of the quality assessment of the included saliva-based studies; (B) Summary of the quality assessment of the included DTS-based studies.

COV, has been seen in the infected rhesus macaque model.[58] Additionally, the secretion of SARS-CoV-1 specific secretory immunoglobulin-A into animal models' saliva has been reported.[59] The third one is indirect viral shedding from blood plasma into the oral cavity inside the gingival crevicular secretions.[60] However, it is known that blood plasma with a detection rate of 7.3% is a minor source of SARS-CoV-2.[7] The last one is the direct infection of oral mucosal endothelial cells via ACE2, a specific receptor of SARS-CoV-1 & 2. Overexpression of ACE2 receptors on the oral cavity mucosa has been stated recently.[61] Any or some of these possible sources may contribute to the viral shedding in the saliva of COVID-19 patients.

Many studies have called specimens collected from the oral cavity or oropharyngeal region saliva despite their different origins and samplings. We categorized the collected samples into pure saliva, saliva, deep throat secretion, and DT-sputum (mentioned as DTS in the text) based on the collected fluids' source and location to maintain evidence consistency. Pure saliva is sampled from the salivary gland orifice. Saliva is the fluid available in the oral cavity. The coughing up/out accompanied by a throat-clearing maneuver can introduce more lower respiratory secretion and sputum into the DT-secretion, resulting in a DT-sputum sample. Therefore, DT-sputum is mixed with the upper and lower respiratory samples and may reveal the sputum specimen's diagnostic potential.[62]

Remarkably, reporting various specimens' different sensitivity has led to significant uncertainty and confusion for diagnosis, estimation of tests' accuracy, and monitoring of SARS-CoV-2 infection. In the present study, we performed a comprehensive systematic review and meta-analysis to compare different upper respiratory specimens' feasibility to detect SARS-CoV-2 RNA. Compared to the previous analysis,[7-10] this study has several advantages, including the classification of specimens by considering saliva-based or sputum-based samples, the use of a large number of studies in the meta-analysis and systematic review, classification and analysis of the findings based on disease severity, analysis of asymptomatic patients, analysis of respiratory diagnostic samples simultaneously with saliva or DT-sputum, evaluation of the relationship between viral load, various samples, and disease severity.

Considering the RT-PCR test's specificity and sensitivity, in vitro analyses demonstrated its high specificity and sensitivity for SARS-CoV-2,[63] despite the questionable SARS-CoV-2 detection rate resulting from upper airway specimens in clinical settings.[51] In this regard, very high specificity and moderate sensitivity (40%-78%) have been estimated for NPS.[8,51] Numerous studies have reported negative results for the NPS, while the saliva or DTS tests positive.[14-16,18,22,23,25,27,33,42] So that, according to current knowledge, none of the approved SARS-COV-2 diagnostic samples is accurate enough to be considered as a gold standard. The WHO and CDC have recommended the lower respiratory tract's sputum as a diagnostic specimen for patients developing a productive cough.[2,47] This sampling is the same as the technique of collecting DTS.[33-35,40,42] Also, the emergency use authorization (EUA) of a saliva-based diagnostic method was approved by the Food and Drug Administration (FDA) in April 2020 for screening.[64] In this review, to maintain consistency in the analysis of evidence, not losing true positive results while the reference test is negative, and also to match the results of different studies with each other, we considered pooled event rates (negative and positive results) of all samples as reference for sensitivity calculation. Furthermore, considering the lack of accurate reference tests and limited available studies to determine false-positive results, specificity calculation was not feasible in the present analysis.

In this study, the highest overall sensitivity for SARS-CoV-2 in symptomatic patients was 97% (95% CI = 86-100) for bronchoalveolar fluid lavage, 92% (95% CI = 80-99) for double naso/oropharyngeal swabs, 87%(95% CI = 77-95) for NPS, 83% (95% CI = 77-89) for saliva, 82% (95% CI = 76-88) for DTS, and 44% (95%CI=35-52) for OPS, based the pooled event rates (Fig. 1-3, Supplementary Material Fig S4). NPS samples showed slightly higher sensitivity than saliva and DTS, although the difference appeared insignificant due to the wide overlap of confidence intervals. OPS revealed the lowest sensitivity, which is consistent with other studies.[8,51] BALF showed the most sensitive means of virological confirmation. However, BALF can only reasonably be collected from critically ill patients.[51]

Ricco et al. reported a detection rate of 83.4% (95% CI = 73.1-90.4) for saliva by analyzing 14 studies, which all are included in our analysis.[10] We found similar sensitivity for OPS and higher sensitivity for NPS and DTS comparing to the estimates reported by Mohammadi et al.[8] They reported 43%(95% CI = 34%-52%) for OPS, 54% (95% CI =41%-67%) for NPS, and 71% (95% CI = 61%-80%) for sputum in a meta-analysis of 11 studies. We found lower sensitivity for sputum and higher sensitivity for saliva than the study from Boger et al, which reported a sensitivity of 0.972 (0.903-0.997) for Sputum and 0.623 (0.545-0.696) for Saliva samples through analyzing limited records.[7] Furthermore, the present study showed lower sensitivity for saliva compared to the meta-analysis of Czumbel et al, which included 4 studies with severe patients (95% CI = 80%-99%).[9] In the present study, considering the method of diagnosis, no difference was found between previously confirmed patients and pooled event rates group

in saliva sample (Supplementary Material Fig S3), which was consistent with the study by Ricco et al.[10]

The sensitivity rate was directly related to the severity of the disease, which means that a positive test's probability increased as the disease's severity increased. The highest sensitivity was obtained from BALF(available in severe patients), double NPS/OPS, saliva, DTS, NPS, and OPS, respectively (Fig. 1-3, Supplementary Material Fig S4). In patients with mild to moderate disease, the highest sensitivity was obtained for double NPS/OPS, NPS, saliva sample, DTS sample, and OPS, respectively. All confidence intervals, except OPS, overlap, indicating that these results are not significantly different. The sensitivity of saliva and DTS for severely ill patients were similar to those found by Czambel et al (91%, 95% CI, 80%-99%) because they only analyzed records of severe patients.[9]

The NPS appeared to be more sensitive in mild patients. According to the subgroup-analysis, the reason was the lack of studies based on the pooled event rates in severe patients compared to mild patients. Hence, to achieve greater consistency in the assessment, we excluded these studies from the group of mild patients, and as a result, the results changed in favor of severe patients.

Overall, the lowest rate of sensitivity was found in asymptomatic patients. However, the number of studies that reported test results on asymptomatic individuals was limited. Saliva showed 83%(95% CI = 77-89) sensitivity in symptomatic patients, approximately two times more than asymptomatic patients (46% with 95% CI = 27-66). The influence of asymptomatic patients on the estimation of overall sensitivity was also evident so that if the number of samples from asymptomatic patients increases, the diagnostic tests' sensitivity decrease. (Supplementary Material Fig S2)

In general, sensitivity was directly related to the viral load so that as the viral load increased, the probability of a positive test increased. Regardless of sample type, several studies found higher viral load and consequently sensitivity in severe patients than mild patients.[16,35,37,41,44,45] So that the mean viral load of severe cases was found around 60 times higher than mild cases.[65] Isolation of the virus has been reported from a considerable fraction of respiratory specimens during the first week from mild patients, whereas no isolates were obtained with a reduction in viral load after the first week.[46] Noteworthy, patients with higher baseline viral load more likely to become severe.[45] In this regard, Magleby et al found that higher viral load was associated with an over 6-fold higher risk of death and a nearly 3-fold higher risk of intubation.[66] Viral load was also highest during the first week after symptom onset and subsequently declined with time,[26,34,35,38,41,46] and it is significantly higher during early and progressive stages than the recovery stage. [34,35,38,41,46] Up to 50% reduction in SARS-CoV-2 detection rates from saliva samples has also been reported during the convalescent period.[18,25] Mohammadi et al found that early sampling following the onset of symptoms was associated with improved detection rates.[8] In this regard, asymptomatic patients show significantly lower viral load and faster viral clearance than symptomatic patients,[15,20] thus less likely to test positive.[20]

Considering viral shedding in the recovery period, NPS showed around three times more sensitivity than saliva.[18] Iwasaki et al reported lower viral load and earlier viral clearance in saliva than NPS[23] (Supplementary Material Table S1). Simultaneously, sputum was slightly more sensitive than NPS in the recovery phase in the study of Yang et al.[37] The sputum has been suggested for patient monitoring and discharge management due to the prolonged viral shedding.[34,35,46] Yoon et al. found higher viral load in sputum than saliva, and saliva was positive only in the first week.[32] Another case is the comparison of the posterior pharyngeal secretions with sputum. Lai et al reported 68%, and Ying Wong et al reported 61% sensitivity using cough-free DT-secretions, while sputum showed 89% sensitivity in paired sampling.[38,39] (Supplementary Material Table S2) It should be noted that the samples of patients who produce sputum showed 2.6 times more sensitivity than non-sputum producers, showing the effect of sputum more.[38]

Based on the current knowledge, the source of saliva contamination is unknown,[67] so sampling from different anatomical sites may lead to different results and sensitivity. Interestingly, sampling from pure saliva revealed only 12.9% positive results, so that 60% of samples from severe patients and 3.8% of mild patients resulted positive.[30] Also, direct sampling from bilateral buccal mucosa showed significantly lower sensitivity and lower viral load in saliva samples than NP swabs in infected children.[31] These findings may indicate a low probability of involvement of the major and minor salivary glands and consequently secretory saliva, and in case of involvement, it occurs in the more severe stages of the disease.[30,31]

Despite considerable heterogeneity in sampling techniques and composition, the present meta-analysis showed similar saliva and DTS sensitivity. It appears that the proximity of the mouth and throat (The latter is where the secretions of the oral cavity, nasopharynx, and lower respiratory tract meet),[34,35] and the constant mixing of the contents of the oral cavity and deep throat through swallowing, clearing the throat, and even during the sample collection procedure, may lead to a similar composition of saliva and DT-sputum.

LIMITATION

Several limitations arising from the structure of analyzed included studies could have been addressed, while none can be sufficiently solved due to the lack of information. We are aware of the potential bias resulting from the insufficient methodological quality of included studies. The high heterogeneity in the analysis of the results was probably caused by differences in samples and origins, sampling techniques, sample number, sampling on different days following symptoms onset, use of antiviral medications, disease severity, RNA shedding and viral load, purification, and diagnostic PCR kits. Other issues that make "an in-depth evaluation" complex and even impossible are the lack of methodological homogeneity, inadequate reporting of methods and outcome parameters, inconsistent quantitative test results, unavailable viral load reports, and inconsistent results in some studies. Also, a significant share of included studied was retrieved from preprint platforms (eg, medrxiv.org). Furthermore, in the lack of an accurate reference test to determine false-positive results, the specificity calculation was not feasible in the present study.

CONCLUSION

The present meta-analysis provides evidence regarding different respiratory specimens. Within the limitation of the present study, saliva and DTS are valuable diagnostic specimens for COVID-19 diagnosis. Self-collected Saliva and DTS as a noninvasive sampling method allow a more facile, cheaper, safer, and broader population screening than current respiratory specimens. They also improve patient acceptance and decrease the risk to health care workers. The results support the use of saliva and DTS as a suitable alternative first-line screening method of SARS-CoV-2 infection based on RT-PCR assay; however, the methods of sampling, storing, and laboratory assay need to be optimized and validated before introducing as an appropriate standardized procedure for definite diagnosis and viral load monitoring in clinical applications. Based on meta-analysis and systematic review of the present study, it seems that the following conclusions can be reached:

- BALF, double NPS/OPS, NPS, saliva, and DTS, showed the highest sensitivity, respectively.
- Saliva, DTS, and NPS showed approximately similar results.

- Double NPS/OPS show higher viral detection than NPS; however, given the 5% difference in diagnosis, a rational and scientific decision is needed to continue to use combined NPS/OPS based on cost and benefit.
- OPS is the most unreliable respiratory sample.
- Viral load and disease severity and SARS-CoV-2 detection rate are directly related.
- Viral load and sensitivity are higher in severe patients than mild patients.
- Viral load and SARS-CoV-2 detection rate are significantly lower, and viral clearance is significantly faster in asymptomatic patients than in symptomatic individuals
- Viral load is highest during the first week after symptom onset and subsequently declined with time.
- None of the diagnostic specimens showed appropriate diagnostic sensitivity in asymptomatic patients.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2021.03.008.

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